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L12: Entry 1 of 50 File: USPT Aug 12, 2003

DOCUMENT-IDENTIFIER: US 6605699 B1
TITLE: Galectin-11 polypeptides

Abstract Text (1):
The present invention relates to galectin 11 proteins which are members of the galectin superfamily. In particular, the present invention relates to full-length polypeptides, fragments, and variants of galectin 11.

Brief Summary Text (2):
The present invention relates to a novel galectin. More specifically, isolated nucleic acid molecules are provided encoding human galectin 11. Galectin 11 polypeptides are also provided, as are vectors, host cells, recombinant methods for producing the same, and antibodies to galectin 11 polypeptides. The invention further relates to screening methods for identifying agonists and antagonists of galectin 11 activity. Also provided are diagnostic methods for detecting cell growth disorders and therapeutic methods for cell growth disorders, including autoimmune diseases, cancer, and inflammatory diseases.

Brief Summary Text (4):
Lectins are proteins that bind to specific carbohydrate structures and can thus recognize particular glycoconjugates. Barondes et al., J. Biol. Chem. 269(33):20807-20810 (1994). Galectins are members of a family of .beta.-galactoside-binding lectins with related amino acid sequences (For review see, Barondes et al., Cell 76:597-598 (1994); Barondes et al., J. Biol. Chem. 269(33):20807-20810 (1994)). Although a large number of glycoproteins containing .beta.-galactoside sugars are produced by the cell, only a few will bind to known galectins in vitro. Such apparent binding specificity suggests a highly specific functional role for the galectins.

Brief Summary Text (5):
Galectin 1 (conventionally termed LGALS1 for lectin, galactoside-binding, soluble -1, but which is also known as: L-14-1, L-14, RL-14.5, galaptin, MGBP, GBP, BHL, CHA, HBP, HPL, HLBP 14, rIML-1) is a homodimer with a subunit molecular mass of 14,500 Daltons. Galectin 1 is expressed abundantly in smooth and skeletal muscle, and to a lesser extent in many other cell types (Couraud et al., J. Biol. Chem. 264:1310-1316 (1989). Galectin 1 is thought to specifically bind laminin, a highly polylactosaminated cellular glycoprotein, as well as the highly polylactosaminated lysosome-associated membrane proteins (LAMPs). Galectin 1 has also been shown to bind specifically to a lactosamine-containing glycolipid found on olfactory neurons and to integrin a.sub.7 b.sub.1 on skeletal muscle cells.

Brief Summary Text (6):
Other members of the Galectin family have also been reported. Galectin 2 was originally found in hepatoma and is a homodimer with a subunit molecular mass of 14,650 Daltons (Gitt et al., J. Biol. Chem. 267.10601-10606 (1992)). Galectin 3 (a.k.a., Mac-2, EPB, CBP-35, CBP-30, and L-29) is abundant in activated macrophages and epithelial cells and is a monomer with an apparent molecular mass between 26,320 and 30,300 Daltons (Cherayil et al., Proc. Natl. Acad. Sci. USA 87: 7324-7326 (1990)). Galectin 3 has been observed to bind specifically to laminin, immunoglobulin E and its receptor, and bacterial lipopolysaccharides. Galectin 4 has a molecular mass of 36,300 Daltons and contains two carbohydrate-binding domains within a single polypeptide chain (Oda et al., J. Biol. Chem. 268:5929-5939 (1993)). Galectins 5 and 6 are discussed in Barondes et al., Cell 76:597-598 (1994). Human

Galectin 7 has a molecular mass of 15,073 Daltons and is found mainly in stratified squamous epithelium (Madsen et al., J. Biol. Chem. 270 (11):5823-5829 (1995)).

Brief Summary Text (7):
Animal lectins, in general, often function in modulating cell-cell and cell-matrix interactions. Galectin 1 has been shown to either promote or inhibit cell adhesion depending upon the cell type in which it is present. Galectin 1 inhibits cell-matrix interactions in skeletal muscle presumably, by galectin 1-mediated disruption of laminin-integrin a.sub.7 b.sub.1 interactions (Cooper et al., J. Cell Biol. 115:1437-1448 (1991)). In several non-skeletal muscle cell types, Galectin 1 promotes cell-matrix adhesion possibly by cross-linking cell surface and substrate glycoconjugates (Zhou et al., Arch. Bioch. Biophys. 300:6-17 (1993); Skrincosky et al., Cancer Res. 53:2667-2675 (1993)).

Brief Summary Text (8):
Galectin 1 also participates in regulating cell proliferation (Wells et al., Cell 64:91-97 (1991)) and some immune functions (Offner et al., J. Neuroimmunol. 28:177-184 (1990)). Galectin 1 induces the release of tumor necrosis factor from macrophages (Kajikawa et al., Life Sci. 39:1177-1181 (1986). Galectin 1 has also been demonstrated to have therapeutic activity against autoimmune diseases in animal models for experimental myasthenia gravis, and experimental autoimmune encephalomyelitis (Levi et al., Eur. J. Immunol. 13:500-507 (1983); and Offner et al., J. Neuroimmunol. 28:177-184 (1990), respectively). Additionally, galectin 1 has been shown to regulate immune response by mediating apoptosis of T cells (Perillo et al., Nature 378:736-739 (1995)).

Brief Summary Text (9):
Galectin 3 promotes the growth of cells cultured under restrictive culture conditions (Yang et al., Proc. Natl. Acad. Sci. USA 93:6737-6742 (June 1996)). Galectin 3 expression in cells confers resistance to apoptosis which indicates that galectin 3 could be a cell death suppresser which interferes in a common pathway of apoptosis. Id. Galectin 3 has also been observed to function in modulating cell-adhesion, as well as in the activation of certain immune cells by cross-linking IgE and IgE receptors.

Brief Summary Text (10):
Recently, a galectin-like antigen designated HOM-HD-21 was found to be highly expressed in a Hodgkin's Disease cDNA library and another galectin, termed PCTA-1, was identified as a specific cell surface marker on human prostate cancer cell lines and patient-derived carcinomas.

Brief Summary Text (11):
Thus, galectins have been observed to be involved in the regulation of immune cell activity, as well as in such diverse processes as cell adhesion, proliferation, inflammation, autoimmunity, and metastasis of tumor cells. Accordingly, there is a need in the art for the identification of novel galectins which can serve as useful tools in the development of therapeutics and diagnostics for regulating immune response, inflammatory disease and cancer.

Brief Summary Text (13):
The present invention provides isolated nucleic acid molecules comprising, or alternatively consisting of, a polynucleotide encoding the galectin 11 polypeptide having the amino acid sequence shown in FIG. 1 (SEQ ID NO:2), the amino acid sequence encoded by the cDNA clone deposited in a bacterial host as ATCC Deposit No. 209053, on May 16, 1997, and fragments, variants, derivatives, and analogs thereof.

Brief Summary Text (14):
The present invention also provides isolated nucleic acid molecules comprising a polynucleotide encoding the galectin 11 polypeptide having the amino acid sequence shown in FIG. 6 (SEQ ID NO:14), referred to herein sometimes as "Galectin-11.alpha." and fragments, variants, derivatives, and analogs thereof.

Brief Summary Text (15):
The present invention also provides isolated nucleic acid molecules comprising a polynucleotide encoding the galectin 11 polypeptide having the amino acid sequence

shown in FIGS. 6A-B (SEQ ID NO:14), referred to herein sometimes as "Galectin-11.alpha." and fragments, variants, derivatives, and analogs thereof.

Brief Summary Text (16):
The present invention also provides isolated nucleic acid molecules comprising a polynucleotide encoding the galectin 11 polypeptide having the amino acid sequence shown in FIGS. 6A-B and 8 (SEQ ID NO:16), referred to herein sometimes as "Galectin-11.beta." and fragments, variants, derivatives, and analogs thereof.

Brief Summary Text (18):
The galectin 11 polynucleotide of FIG. 1 (SEQ ID NO:1), the galectin 11.alpha. polynucleotide of FIGS. 6A-B (SEQ ID NO:24), and the galectin 11.beta. polynucleotide of FIG. 7 (SEQ ID NO:26) are often referred to herein collectively as, e.g., "galectin 11 polynucleotides."

Brief Summary Text (19):
The present invention also relates to recombinant vectors which include the isolated nucleic acid molecules of the invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and for using them for production of galectin 11 polypeptides by recombinant techniques.

Brief Summary Text (20):
The invention further provides isolated galectin 11 polypeptides, including galectin 11 of SEQ ID NO:2 and galectin 11.alpha. and .beta., having an amino acid sequence encoded by a polynucleotide described herein and antibodies which bind these polypeptides. The galectin 11 polypeptide of FIG. 1 (SEQ ID NO:2), the galectin 11.alpha. polypeptide of FIGS. 6A-B (SEQ ID NO:25), and the galectin 11.beta. polypeptide of FIG. 7 (SEQ ID NO:27) are often referred to herein collectively as, e.g., "galectin 11 polypeptides."

Brief Summary Text (21):
The present invention also provides screening methods for identifying compounds capable of enhancing or inhibiting a cellular response, such as, for example, apoptosis, induced by galectin 11. Generally, these methods involve contacting galectin 11, the candidate compound, and a cell which expresses a galectin 11 ligand, assaying a cellular response resulting from the binding of galectin 11 with the ligand, and comparing the cellular response to a standard, the standard being assayed when contact of galectin 11 and the galectin 11 ligand is made in the absence of the candidate compound; whereby, an increased cellular response over the standard indicates that the compound is an agonist and a decreased cellular response over the standard indicates that the compound is an antagonist.

Brief Summary Text (23):
The invention also provides diagnostic methods useful during diagnosis of disorders associated with elevated, decreased, or otherwise aberrant expression of galectin 11.

Brief Summary Text (24):
The invention further provides for methods for treating an individual in need of an increased level of galectin 11 activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount of an isolated galectin 11 polypeptide, fragment, variant, derivative, or analog of the invention, or an agonist thereof.

Drawing Description Text (2):
FIG. 1 shows the nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of galectin 11. The protein has a deduced molecular mass of about 14.8 kDa. The complementary strand of the nucleotide sequence of SEQ ID NO:1 is shown in SEQ ID NO:12.

Drawing Description Text (3):
FIG. 2 shows the regions of similarity between the amino acid sequences of the galectin 11 protein (HJACE54) (SEQ ID NO:2), rat galectin 5 (SEQ ID NO:3), and human galectin 8 (SEQ ID NO:4). Identical amino acids shared between the galectins are

shaded, while conservative amino acid changes are boxed. By examining the regions of amino acids shaded and/or boxed, the skilled artisan can readily identify conserved domains between the two polypeptides. These conserved domains are preferred embodiments of the present invention.

Drawing Description Text (4):
FIG. 3 shows structural and functional features of galectin 11 (SEQ ID NO:2) predicted using the default parameters of the indicated computer programs. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the Antigenic. Index--Jameson-Wolf graph, the positive peaks indicate locations of the highly antigenic regions of the galectin 11 protein, i.e., regions from which epitope-bearing peptides of the invention can be obtained. The domains defined by these graphs are contemplated by the present invention, including for example, amino acid residues 65-70 and 118-124 in FIG. 1 (SEQ ID NO:2), which correspond to the shown highly antigenic regions of the galectin 11 polypeptide.

Drawing Description Text (7):
FIG. 5A is a bar graph showing that transfection of Jurkat cells with a galectin 11 expression construct (pEF-Leg11) induces apoptosis of transfected cells. Shaded bars represent % apoptosis of Jurkat cells that have been transfected with the galectin 11 expression construct, whereas open bars represent % apoptosis of Jurkat cells that have been transfected with the pEF control vector. Apoptosis was measured by two-color cytometry using mitoTracker Red.

Drawing Description Text (8):
FIG. 5B is a bar graph showing the survival of GFP positive cells that have been successfully transfected, 4 days after transfection. The survival of the transfected cells was examined after co-transfection with either the control vector (pEF 1), or the galectin 11 expression vector (pEF-Leg11). There were about 4 times more surviving GFP positive cells after transfection with pEF1 than with pEF-Leg11.

Drawing Description Text (9):
FIGS. 6A-B shows the nucleotide sequence (SEQ ID NO:24) and deduced amino acid sequence (SEQ ID NO:25) of the complete galectin 11.alpha. cDNA and protein, respectively.

Drawing Description Text (13):
The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding a galectin 11 polypeptide having the amino acid sequence shown in FIG. 1 (SEQ ID NO:2), FIGS. 6A-B (SEQ ID NO:25), or FIGS. 6A-B and 8 (SEQ ID NO:27) which were determined by sequencing cloned cDNAs. The nucleotide sequence shown in FIG. 1 (SEQ ID NO:1) was obtained by sequencing the HJACE54 plasmid which was deposited on May 16, 1997 at the American Type Culture Collection, 10801 University Boulevard, Manassas, Va., and given accession number 209053. The galectin 11 polypeptides of the present invention share sequence homology with rat galectin 5, chicken galectin 3, and human galectin 8 gene products (see, e.g., FIG. 2; SEQ ID NOS: 3-4).

Drawing Description Text (14):
The invention further provides for fragments, variants, derivatives and analogs of galectin 11 polynucleotides and polypeptides encoded thereby, and antibodies which bind these polypeptides.

Drawing Description Text (17):
"Functional activity" or "biological activity" refers to galectin 11 polypeptides, fragments, derivatives, variants, and analogs, exhibiting activity similar, but not necessarily identical to, an activity of a galectin 11 polypeptide, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the galectin 11 polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the galectin 11 polypeptide (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the

galectin 11 polypeptide.) Such functional activities include, but are not limited to, biological activity (such as, for example, the ability to bind a .beta.-galactoside sugar, the ability to agglutinate trypsin-treated rabbit erythrocytes and/or to induce apoptosis), antigenicity (ability to bind or compete with a galectin 11 polypeptide for binding to an anti-galectin 11 antibody), immunogenicity (ability to generate antibody which binds to a galectin 11 polypeptide), the ability to form dimers with galectin 11 polypeptides of the invention, and the ability to bind to other galectins and/or a receptor or ligand for galectin 11. Polynucleotides encoding polypeptides having galectin 11 functional or biological activity, and the complementary strand of these polynucleotides are also encompassed by the invention.

Drawing Description Text (18):
"Polynucleotide" generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA, or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications have been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

Drawing Description Text (19):
"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl tennini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given galectin 11 polypeptide. Also, a given galectin 11 polypeptide may contain many types of modifications. Galectin 11 polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS--STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter et al., "Analysis for protein modifications and nonprotein cofactors", Meth Enzymol 182:626-646 (1990) and Rattan et al., "Protein Synthesis: Posttranslational Modifications and Aging", Ann NY Acad

Sci 663:48-62 (1992).)

Drawing Description Text (20):
"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains functional or biological activity of galectin 11. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

Drawing Description Text (24):
Therefore, SEQ ID NO:1 and the translated SEQ ID NO:2 are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:1 is useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:1 or the cDNA contained in the deposited clone. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:2 may be used, for example, to generate antibodies which bind specifically to proteins galectin 11.

Drawing Description Text (26):
Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:1 and the predicted translated amino acid sequence identified as SEQ ID NO:2, but also a sample of plasmid DNA containing a human cDNA of galectin 11 deposited with the ATCC. The nucleotide sequence of the deposited galectin 11 clone can readily be determined by sequencing the deposited clone in accordance with known methods. The predicted galectin 11 amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by the deposited clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human galectin 11 cDNA, collecting the protein, and determining its sequence.

Drawing Description Text (27):
Using the information provided herein, such as the nucleotide sequence in FIG. 1, a nucleic acid molecule of the present invention encoding a galectin 11 polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the nucleic acid molecule described in FIG. 1 (SEQ ID NO:1) was discovered in a cDNA library derived from G1 phase Jurkat T-cells. This gene was also identified in cDNA libraries generated from human neutrophil and human infant adrenal gland. Polynucleotides of the invention can also be obtained from natural sources such as mRNA or genomic DNA using techniques known in the art, or can be chemically synthesized using techniques known in the art.

Drawing Description Text (28):
The human galectin 11 gene is located on chromosome 11 and contains 5 exons (see, e.g., FIG. 4). The nucleotide sequence of the galectin 11 cDNA of FIG. 1 (SEQ ID NO:1) is 865 nucleotides in length (830 nucleotides discounting the poly A tail of the cDNA) which encodes a predicted open reading frame of 133 amino acid residues.

There is a predicted initiation codon at nucleotides 49-51 of the nucleotide sequence depicted in FIG. 1 (SEQ ID NO:1), located on the second exon of the gene. The galectin 11 protein shown in FIG. 1 (SEQ ID NO:2) shares homology with the translation product of rat galectin 5, chicken galectin 3, and human galectin 8 (see, e.g., FIG. 2). Additionally, as further discussed below, galectin 11 induces apoptosis of transfected T-cells (see Example 5 and FIGS. 5A and 5B). These findings indicate that galectin 11 functions in a manner similar to other previously characterized galectins and therefore, that galectin 11 is important in the regulation of cell growth disorders, autoimmune diseases, cancer, and inflammatory diseases.

Drawing Description Text (29):
The nucleotide sequence of the galectin 11 cDNA of FIGS. 6A-B (SEQ ID NO:24) is 1337 nucleotides in length. This is one of two alternatively spliced forms of galectin 11 and is referred to as galectin 11.alpha.. The other form, galectin 11.beta., differs only in the loss of 7 nucleotides (nucleotides 136-142 as shown in FIGS. 6A-B (SEQ ID NO:24)). See FIG. 7. The sequence of galectin 11.beta. is shown in the sequence listing as SEQ ID NO:26. The resulting translation products of these splice variants are believed to differ only at the N-terminus. The amino acid sequences of galectin 11.alpha. and .beta. are shown in the sequence listing as SEQ ID NOS:25 and 27, respectively. The differences between the two proteins are highlighted in FIG. 8.

Drawing Description Text (30):
The galectin 11 polypeptide is comprised of two carbohydrate binding domains (CARD domains) separated by a linker sequence. The first carbohydrate binding domain consists of the first 121 amino acid residues of galectin-11.alpha. (SEQ ID NO:25) and the first 142 amino acids of galectin 11.beta. (SEQ ID NO:27). The 29 amino acid residues following the first CARD domain is the linker sequence. Finally, the last 125 amino acid residues in each protein is the C-terminal CARD domain. Preferred polypeptides of the invention comprise either an N-terminal or C-terminal CARD domain. Polynucleotides encoding such polypeptides are also provided.

Drawing Description Text (31):
Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologs of genes corresponding to SEQ ID NO:1-2, 24-25, 26-27, or the deposited clone, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.

Drawing Description Text (32):
As one of ordinary skill would appreciate, due to the possibilities of sequencing errors discussed above, as well as the variability of processing sites for different known proteins, the predicted galectin 11 polypeptide encoded by the deposited cDNA comprises about 133 amino acid residues, but may be anywhere in the range of 125-150 amino acids.

Drawing Description Text (34):
By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution. Isolated RNA molecules include in vivo or in vitro RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically. In a specific embodiment, "isolated" nucleic acid molecules of the invention comprise all or a portion of the coding region of galectin 11, as disclosed in FIG. 1 (SEQ ID NO:1) or galectin 11.alpha. as disclosed in FIGS. 6A-B (SEQ ID NO:24), or galectin 11.beta. as disclosed in SEQ ID NO:26. The term "isolated" does not refer to genomic

or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

Drawing Description Text (35):
Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) or a portion of an ORF shown in FIG. 1 or 6A-B (SEQ ID NO:1, 24, or 26); and DNA molecules which comprise a sequence substantially different from those described above, but which due to the degeneracy of the genetic code, still encode the galectin 11 protein. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate variants.

Drawing Description Text (36):
In specific embodiments, the invention provides isolated nucleic acid molecules encoding the full length galectin 11 polypeptide depicted in FIG. 1 (SEQ ID NO:2), and galectin 11 nucleic acid molecules encoding the galectin 11 polypeptide sequence encoded by the cDNA clone contained in the plasmid deposited as ATCC Deposit No. 209053, on May 16, 1997. In a further embodiment, nucleic acid molecules are provided encoding the full length galectin 11 polypeptide lacking the N-terminal methionine. The invention further provides an isolated nucleic acid molecule having the nucleotide sequence shown in FIG. 1 (SEQ ID NO:1) or the nucleotide sequence of the galectin 11 cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, have uses which include, but are not limited to, probes for gene mapping by in situ hybridization with chromosomes, and for detecting expression of the galectin 11 gene in human tissue, for instance, by Northern blot analysis. The invention further provides a polynucleotide encoding a polypeptide comprising the full-length amino acid sequence shown as SEQ ID NO:25 or 27, with or without an N-terminal methoinine.

Drawing Description Text (37):
In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5 kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the galectin 11 gene of interest on chromosome 11). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 1, 5, 4, 3, 2, or 1 genomic flanking gene(s).

Drawing Description Text (38):
The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated nucleic acid molecule having the nucleotide sequence of the deposited cDNA, the nucleotide sequence shown in FIGS. 1 and 6A-B (SEQ ID NOS:1, 24, and 26), or the complementary strand thereto, is intended fragments of at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length. By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of the deposited cDNA or the nucleotide sequence as shown in FIG. 1 (SEQ ID NO:1) or the cDNA shown in FIG. 6 (SEQ ID NOS:24 and 26) or the complementary strand thereto. Also encompassed by the invention are DNA fragments comprising 50, 100, 150, 200, 250, 300, 350, 365, 370, 375, 380, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850 contiguous nucleotides of the sequence shown in FIG. 1 (SEQ ID NO:1), the strand complementary thereto, or contained in the deposited clone. The present invention also encompasses fragments corresponding to most, if not all, of the nucleotide sequence of the deposited cDNA or as shown in FIG. 1 (SEQ ID NO:1) or the complimentary strand thereto. In further embodiments, the polynucleotide fragments of the invention comprise a sequence which encodes amino acids 1-14, 1-20, 1-40,

1-66, 2-67, 3-8, 3-67, 5-108, 5-128, 10-17, 10-20, 12-16, 13-20, 13-68, 14-67, 23-40, 20-50, 40-108, 41-60, 47-61, 47-108, 47-128, 50-100, 61-80, 65-108, 65-128, 66-108, 76-88, 81-100, 88-108, 88-128, 95-101, 101-133, 108-120, 114-128, and/or 114-128 of the amino acid sequence depicted in FIG. 1 (SEQ ID NO:2). In preferred embodiments, polynucleotide fragments of the invention encode a polypeptide which demonstrates a galectin 11 functional activity. Fragments of the invention have numerous uses which include, but are not limited to, diagnostic probes and primers as discussed herein.

Drawing Description Text (39):
Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding epitope-bearing portions of the galectin 11 protein. In particular, such nucleic acid fragments of the present invention include nucleic acid molecules encoding: a polypeptide comprising amino acid residues from about 65-70 and 118-124 in FIG. 1 (SEQ ID NO:2). The inventors have determined that the above polypeptide fragments are antigenic regions of the galectin 11 protein. Methods for determining other such epitope-bearing portions of the galectin 11 protein are described in detail below.

Drawing Description Text (40):
In other embodiments, the invention provides an isolated nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide which hybridizes under stringent hybridization conditions to all or a portion of a galectin 11 polynucleotides (including fragments) described herein, the complementary strand thereof, the cDNA clone contained in ATCC Deposit No. 209053, on May 16, 1997, or fragments thereof. By "stringent hybridization conditions" is intended overnight incubation at 42.degree. C. in a solution comprising: 50% formamide, 5.times.SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5.times.Denhardt's solution, 10% dextran sulfate, and 20 .mu.g/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1.times.SSC at 65.degree. C.

Drawing Description Text (41):
Also contemplated are nucleic acid molecules that hybridize to the galectin 11 polynucleotides under lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C. in a solution comprising 6.times.SSPE (20.times.SSPE=3M NaCl; 0.2M NaH.sub.2 PO.sub.4 ; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 .mu.g/ml salmon sperm blocking DNA; followed by washes at 50 degree C. with 1.times.SSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5.times.SSC).

Drawing Description Text (43):
By a polynucleotide which hybridizes to a portion of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20, still more preferably at least about 30, 50, 60, 75, 100, 150, 175, 200, 250, 300, 350 nt preferable about 30-70 nt, or 80-150 nucleotides, or the entire length of the reference polynucleotide. By a portion of a polynucleotide of at least "20 nt in length", for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the deposited cDNA or the nucleotide sequence as depicted in FIG. 1 (SEQ ID NO:1). In specific embodiments, the polynucleotide hybridizes to nucleotides 0-20, 0-25, 0-30, 0-50, 51-100, 80-100, 101-200, 201-300, 301-400, 401-450, 451-500, 501-550, 551-600, 601-700, 701-750, 751-780, and/or 780-820 of the nucleotide sequence disclosed in FIG. 1 (SEQ ID NO:1). In other specific embodiments, the polynucleotide hybridizes to a nucleotide sequence which encodes amino acid residues 1-14, 10-20, 20-50, 50-100, 100-133 of the amino acid sequence depicted in FIG. 1 (SEQ ID NO:2). In specific embodiments, the polynucleotide hybridizes to nucleotides 1-20, 1-25, 1-30, 1-50, 51-100, 80-100, 101-200, 201-300, 301-400, 401-450, 451-500, 501-550, 551-600, 601-700, 701-750, 751-800, 801-850, 851-900, 901-950, 951-1,000, 1,001-1050, 1,051-1,100, 1,101-1,150, 1,151-1,200, 1,201-1,250, and/or 1,251-1,337 of the nucleotide sequence disclosed in SEQ ID NO:24. In other specific embodiments, the polynucleotide hybridizes to a nucleotide

sequence which encodes amino acid residues 1-14, 10-20, 20-50, 50-100, 100-130, 130-160, 160-210, 210-240 and/or 240-275 of the amino acid to sequence depicted in SEQ ID NO:25. In specific embodiments, the polynucleotide hybridizes to nucleotides 1-20, 1-25, 1-30, 1-50, 51-100, 80-100, 101-200, 201-300, 301-400, 401-450, 451-500, 501-550, 551-600, 601-700, 701-750, 751-800, 801-850, 851-900, 901-950, 951-1,000, 1,001-1050, 1,051-1,100, 1,101-1,150, 1,151-1,200, 1,201-1,250, and/or 1,251-1,330 of the nucleotide sequence disclosed in FIG. SEQ ID NO:26. In other specific embodiments, the polynucleotide hybridizes to a nucleotide sequence which encodes amino acid residues 1-14, 10-20, 20-50, 50-100, 100-130, 130-160, 160-210, 210-240, 240-270 and/or 270-296 of the amino acid sequence depicted in SEQ ID NO:27. These polynucleotides have uses which include, but are not limited to, diagnostic probes and primers, as discussed above and in more detail below.

Drawing Description Text (44):
Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3' terminal poly(A) tract of the galectin 11 cDNA shown in FIG. 1 (SEQ ID NO:1), FIGS. 6A-B (SEQ ID NO:24) or SEQ ID NO:26 or to a complementary stretch of T (or U) residues, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using an oligo-dT primer).

Drawing Description Text (45):
As indicated, nucleic acid molecules of the present invention which encode a galectin 11 polypeptide may include, but are not limited to, those encoding the amino acid sequence of the polypeptide, by itself; the coding sequence for the polypeptide and additional sequences, such as those encoding an amino acid leader or secretory sequence, such as a pre-, or pro- or prepro-protein sequence; the coding sequence of the polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to, introns and non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals, for example--ribosome binding and stability of mRNA; an additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities. Thus, the sequence encoding the polypeptide may be fused to a marker sequence, such as a sequence encoding a peptide which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (Qiagen, Inc.), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The "HA" tag is another peptide useful for purification which corresponds to an epitope derived from the influenza hemagglutinin protein, which has been described by Wilson et al., Cell 37:767-778 (1984). As discussed below, other such fusion proteins include the galectin 11 fused to Fc at the N- or C-terminus.

Drawing Description Text (46):
The present invention is also directed to polynucleotide fragments of the polynucleotides of the invention. In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence which: is a portion of that contained in a deposited clone, or encoding the polypeptide encoded by the cDNA in a deposited clone; is a portion of that shown in SEQ ID NO:1, 24, or 26 or the complementary strand thereto, or is a portion of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2, 25, or 27. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in a deposited clone or the nucleotide sequence shown in SEQ ID NO:1, 24, or 26. In this context "about" includes the particularly recited value, a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. These nucleotide fragments have uses that include, but are not limited to,

as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500, 600, 2000 nucleotides) are preferred.

Drawing Description Text (47):
Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-48, 49-99, 100-150, 151-201, 202-252, 253-303, 304-354, 355-405, 406-450, 451-501, and 502 to the end of SEQ ID NO:1, or the complementary strand thereto, or the cDNA contained in the deposited clone. In this context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity. More preferably, these polynucleotides can be used as probes or primers as discussed herein. Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

Drawing Description Text (49):
However, many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:1 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 851 of SEQ ID NO:1, b is an integer of 15 to 865, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:1, and where the b is greater than or equal to a+14.

Drawing Description Text (50):
The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode a portion (i.e., fragments), analogs or derivatives of the galectin 11 protein. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

Drawing Description Text (51):
Such variants include those produced by nucleotide substitutions, deletions or additions which may involve one or more nucleotides Particularly preferred are variants in which the nucleotide sequence encoding 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 15, 20, 25, 30, 35, 40, 50, or, 20-15, 15-10, 10-5 1-5, 1-3, or 1-2 amino acids of a polypeptide of the invention are substituted, deleted, or added in any combination. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletion, which do not alter the properties and activities of the galectin 11 protein or portions thereof. Also especially preferred in this regard are conservative substitutions.

Drawing Description Text (52):
Further embodiments of the invention include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 75%, 80%, 85%, or 90% identical, and more preferably at least 95%, 96%, 97%, 98% or 99% or 98-99% identical to (a) a nucleotide encoding amino acids 1 to 133 of SEQ ID NO:2; (b) a nucleotide encoding amino acids 2 to 133 of SEQ ID NO:2; (c) a nucleotide sequence of the galectin 11 polypeptide encoded by the cDNA contained in ATCC Deposit No. 209053; (d) a nucleotide encoding amino acids 1 to 275 of SEQ ID NO:25; (e) a nucleotide encoding amino acids 1 to 296 of SEQ ID NO:27; (f) a nucleotide encoding amino acid residues 1 to 121 of SEQ ID NO:25; (g) a nucleotide encoding amino acid residues 1 to 142 of SEQ ID NO:27; (h) a nucleotide encoding amino acids 2 to 275 of SEQ ID NO:25; (i) a nucleotide encoding amino acid residues 2 to 296 of SEQ ID NO:27; (j) a nucleotide encoding amino acids 151 to 275 of SEQ ID NO:25; or (k)

fragments and other polynucleotide sequences of the invention as described herein. Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

Drawing Description Text (53):
By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a galectin 11 polypeptide of the present invention is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five nucleotide mismatches per each 100 nucleotides of the reference nucleotide sequence encoding the galectin 11 polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The query sequence may be an entire sequence shown of SEQ ID NO:1, the ORF (open reading frame), or any fragment specified as described herein.

Drawing Description Text (58):
The galectin 11 variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Galectin 11 polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as E. coli).

Drawing Description Text (59):
Naturally occurring galectin 11 variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Drawing Description Text (60):
Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the galectin 11 polypeptides. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. The authors of Ron et al., J. Biol. Chem. 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

Drawing Description Text (61):
Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than

3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Drawing Description Text (62):
Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Drawing Description Text (64):
The present application is directed to nucleic acid molecules at least 75%, 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences disclosed herein (e.g., nucleic acid sequence shown in FIG. 1 or 6A-B (SEQ ID NO:1, 24, or 26), nucleic acid sequence of the deposited cDNA clone, and nucleic acid sequences encoding a polypeptide having the amino acid sequence of an N and/or C terminal deletion disclosed below as m-n of SEQ ID NO:2, 25, or 27), irrespective of whether they encode a polypeptide having galectin 11 functional activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having galectin 11 functional activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having galectin 11 functional activity include, inter alia, (1) isolating the galectin 11 gene or allelic or splice variants thereof in a cDNA library; (2) in situ hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the galectin 11 gene, as described in Verma et al., Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York (1988); (3) use in linkage analysis as a marker for chromosome 11; and (4) Northern Blot analysis for detecting galectin 11 mRNA expression in specific tissues.

Drawing Description Text (65):
Preferred, however, are nucleic acid molecules having sequences at least 75%, 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence disclosed herein, shown in FIG. 1 or 6A-B (SEQ ID NO:1, 24, or 26), nucleic acid sequence of the deposited cDNA clone, the nucleic acid encoding the polypeptide shown in FIG. 1 or 6A-B (SEQ ID NO:2, 25, or 27), and fragments thereof, which do, in fact, encode a polypeptide having galectin 11 functional activity. By "a polypeptide having galectin 11 functional activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to a functional activity of the galectin 11 protein of the invention (e.g., complete (full-length) galectin 11, and mature galectin 11), as measured in a particular assay. For example, galectin 11 protein activity can be measured using a .beta.-galactoside sugar (e.g., thiodigalactoside or lactose) binding assay, an assay for apoptosis and/or an assay for agglutination of trypsin-treated rabbit erythrocytes, as further described below.

Drawing Description Text (66):
Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 75%, 80%, 85%, 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of the deposited cDNA, the nucleic acid sequence shown in FIG. 1 or 6A-B (SEQ ID NO:1, 24, or 26), the nucleic acid encoding the polypeptide shown in FIG. 1 or 6A-B (SEQ ID NO:2, 25, or 27), or fragment thereof, will encode "a polypeptide having galectin 11 functional activity". In fact, since numerous degenerate variants of these nucleotide sequences encode the same polypeptide, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having galectin 11 activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less

likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below.

Drawing Description Text (67):
For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions, Science 247:1306-1310 (1990), wherein the authors indicate that proteins are surprisingly tolerant of amino acid substitutions.

Drawing Description Text (68):
The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

Drawing Description Text (69):
The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

Drawing Description Text (70):
As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and GIn, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

Drawing Description Text (75):
For example, galectin 11 polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2-331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

Drawing Description Text (78):
Additionally, more than one amino acid (e.g., 2, 3, 4, 5, 6, 7, 8, 9 and 10) can be replaced with the substituted amino acids as described above (either conservative or nonconservative). The substituted amino acids can occur in the full length, mature, or proprotein form of galectin 11 protein, as well as the N- and C-terminal deletion mutants, having the general formula m-n, [m.sup.1 -n.sup.1, m.sup.1 --n.sup.2, m.sup.1 -n.sup.3, m.sup.2 -n.sup.1, m.sup.2 -n.sup.2, m.sup.2 -n.sup.3, m.sup.3 -n.sup.1, m.sup.3 -n.sup.2 and m.sup.3 -n.sup.3].

Drawing Description Text (80):
Vectors, Host Cells and Protein Production

Drawing Description Text (81):

The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the polynucleotides and/or recombinant vectors of the invention, and the production of galectin 11 polypeptides and fragments, variants, derivatives, and analogs thereof, by recombinant techniques.

Drawing Description Text (82):
Galectin 11 polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

Drawing Description Text (85):
As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase or G418 neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in E. coli and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as E. coli, Streptococcus staphylococci, Bacillus subtilis, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Drawing Description Text (86):
Selection of appropriate vectors and promoters for expression in a host cell is a well known procedure and the requisite techniques for expression vector construction, introduction of the vector into the host, and expression in the host are routine skills in the art. A great variety of expression vectors can be used to express galectin 11 polypeptides and fragments, variants, derivatives, and analogs of the invention. Such vectors include chromosomal, episomal and virus-derived vectors e.g., vectors derived from bacterial plasmids, from bacteriophage, from yeast episomes, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids, all may be used for expression in accordance with this aspect of the present invention. Generally, any vector suitable to maintain, propagate or express polynucleotides to express a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression vector system by any of a variety of known technique, such as for example, those set forth in Ausubel et al., eds., 1989, Current Protocols in Molecular Biology, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., New York.

Drawing Description Text (88):
The present invention also relates to host cells containing the vector constructs discussed herein, and additionally encompasses host cells containing nucleotide sequences of the invention that are operably associated with one or more heterologous control regions (e.g., promoters and/or enhancers) using techniques known in the art. As discussed above, the host cell can be a higher eukaryotic cell, such as a mammalian cell (e.g., a human derived cell), or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. The host strain may be chosen which modulates the expression of the inserted gene sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus expression of the genetically engineered polypeptide may be controlled. Furthermore, different host cells have characteristics and specific mechanisms for the translational and post-translational processing and modification (e.g., glycosylation, phosphorylation, cleavage) of proteins. Appropriate cell lines can be chosen to ensure the desired modifications and processing of the foreign protein expressed.

Drawing Description Text (89):
For secretion of the translated protein into the lumen of the endoplasmic reticulum,

into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide using techniques known in the art. These signals may be endogenous to the polypeptide or they may be heterologous signals.

Drawing Description Text (90):
Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that galectin 11 polypeptides may in fact be expressed by a host cell lacking a recombinant vector.

Drawing Description Text (91):
The polypeptide may be expressed in a modified form, such as a fusion protein (comprising the polypeptide joined via a peptide bond to a heterologous protein sequence (of a different protein)), and may include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. Alternatively, such a fusion protein can be made by protein synthetic techniques, e.g., by use of a peptide synthesizer.

Drawing Description Text (92):
A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to solubilize proteins. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when Fc portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as antigen for immunizations. In drug discovery, for example, human proteins, such as, hIL5- has been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, Bennett et at., J. Md. Recog. 8:52-58 (1995) and Johanson et al., J. Biol. Chem. 270(16):9459-9471 (1995).

Drawing Description Text (93):
The galectin 11 protein can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Drawing Description Text (94):
Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, plant, insect, teleost, avian, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue or may be missing an initial methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any

protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

Drawing Description Text (95):
In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., galectin 11 coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with galectin 11 polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous galectin 11 polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous galectin 11 polynucleotide sequences via homologous recombination, resulting in the formation of a new transcription unit (see, e.g., U.S. Pat. No. 5,641,670, issued Jun. 24, 1997; U.S. Pat. No. 5,733,761, issued Mar. 31, 1998; International Publication No. WO 96/29411, published Sep. 26, 1996; International Publication No. WO 94/12650, published Aug. 4, 1994; Koller et at., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

Drawing Description Text (96):
In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W. H. Freeman & Co., N.Y., and Hunkapiller et al., Nature, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a galectin 11 polypeptide can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the galectin 11 polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

Drawing Description Text (98):
Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Drawing Description Text (100):
The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000,

16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

Drawing Description Text (102):
The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., Exp. Hematol. 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

Drawing Description Text (103):
As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

Drawing Description Text (104):
One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

Drawing Description Text (105):
As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado et al., Crit. Rev. Thera. Drug Carrier Sys. 9:249-304 (1992); Francis et al., Intern. J. of Hematol. 68:-118 (1998); U.S. Pat. No. 4,002,531;

Drawing Description Text (106):
U.S. Pat. No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference. One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride (CISO.sub.2 CH.sub.2 CF.sub.3). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoreothane sulphonyl group.

Drawing Description Text (107):
Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Pat. No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1 '-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention. The number of polyethylene glycol moieties attached to each protein of the invention (i.e., the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2--4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado et al., Crit. Rev. Thera. Drug Carrier Sys. 9:249-304 (1992).

Drawing Description Text (108):
The galectin 11 polypeptides of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the galectin 11 polypeptides of the invention, their preparation, and compositions (preferably, Therapeutics) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers. Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the amino acid sequence of SEQ ID NO:2, or alternatively SEQ ID NO:25 or 27, or encoded by the cDNA contained in the deposited clone (including fragments, variants, splice variants, and fusion proteins, corresponding to these as described herein). These homomers may contain galectin 11 polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only galectin 11 polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing galectin 11 polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (e.g., containing galectin 11 polypeptides having identical or different amino acid sequences) or a homotrimer (e.g., containing galectin 11 polypeptides having, identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

Drawing Description Text (109):
As used herein, the term heteromer refers to a multimer containing, one or more heterologous polypeptides (i.e., polypeptides of different proteins) in addition to the galectin 11 polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Drawing Description Text (110):
Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion

protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the galectin 11 polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in SEQ ID NO:2, 25, or 27, or contained in the polypeptide encoded by the clone HJACE54). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a galectin 11 fusion protein. In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., U.S. Pat. No. 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in a galectin 11-Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is to capable of forming covalently associated multimers, such as for example, oseteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Drawing Description Text (111):
Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

Drawing Description Text (112):
Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

Drawing Description Text (113):
In further preferred embodiments, Galectin 11 polynucleotides of the invention are fused to a polynucleotide encoding a "FLAG" polypeptide. Thus, a galectin 11-FLAG fusion protein is encompassed by the present invention. The FLAG antigenic polypeptide may be fused to an galectin 11 polypeptide of the invention at either or both the amino or the carboxy terminus. In preferred embodiments, a galectin 11-FLAG fusion protein is expressed from a pFLAG-CMV-5a or a pFLAG-CMV-1 expression vector (available from Sigma, St. Louis, Mo., USA). See, Andersson, S., et al., J. Biol. Chem. 264:8222-29 (1989); Thomsen, D. R., et al., Proc. Natl. Acad. Sci. USA, 81:659-63 (1984); and Kozak, M., Nature 308:241 (1984) (each of which is hereby incorporated by reference). In further preferred embodiments, a galectin 11-FLAG fusion protein is detectable by anti-FLAG monoclonal antibodies (also available from Sigma).